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Phosphorus effects on radial oxygen loss, root porosity and iron plaque in two mangrove seedlings under cadmium stress

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ABSTRACT

Phosphorus is an indispensable element for plants, but its role in alleviating the cadmium toxicity of mangrove seedlings is poorly documented. In this study, mangrove seedlings were grown in hydroponics and exposed to various Cd and P treatments. Data suggested that the inhibitory effect of Cd on the rate of radial oxygen loss and root porosity was alleviated by P. *A. marina* had a higher rate of ROL and POR, indicating that it had a stronger adaptability to anaerobic environment. *K. obovata* induced a higher Fe concentration in iron plaque under co-application of Cd and P, which may relate to higher biomass. Furthermore, P increased Cd concentration in iron plaque, implying that iron plaque can be an obstacle to prevent Cd entering into the plant, but most Cd was still distributed in its roots. These findings highlight a novel mechanism of Cd detoxification with P addition in mangrove seedlings.

1. Introduction

Mangrove plants are important parts of the mangrove ecosystem, distributed in the ecotone of terrestrial ecosystem and marine ecosystem. They play an important role in maintaining the balance of coastal ecosystems (Lee et al., 2014). Mangrove plants often grow in muddy beaches, slow-flowing streams, high organic matter and high H_2S reducing habitats. These environmental characteristics enable mangroves to be a potential heavy metal enrichment regions (Liu et al., 2015). In recent years due to aquaculture, urbanization and coastal landfill, mangrove plants have directly or indirectly, suffered varying degrees of pollution (Zhang et al., 2014; Nowrouzi et al., 2012). Li et al. (2015) studying heavy metal pollution and ecological risks in mangrove forests of China, found that cadmium pollution presented a severe threat. Cadmium (Cd) is considered as a nonessential element and can be readily absorbed and accumulated in plants. It can damage membrane structure, decrease plant's respiration rate, inhibit photosynthesis, lead to nutrient absorption imbalance and growth retardation or enter the food chain (Nagajyoti et al., 2010; Xue et al., 2017).

Phosphorus (P) is one of the basic and important elements of the estuarine environment. It is also an important component of organic compounds for the growth of mangrove plants distributed at the intertidal zone. However, the sedimentary environment of mangrove wetland presents a lack of oxygen and soluble toxins accumulations as well as low nutrient availability. Many studies suggest that P is one of the main factors influencing the mangrove forest structure and productivity (Inoue et al., 2011; Reef et al., 2010). At the same time, deficiency of oxygen can cause nutrient deficiency due to decreasing nutrient absorption, resulting in plant senescence and growth inhibition (Bar-Yosef and Lieth, 2013).

In order to overcome these environmental threats and be able to withstand the harsh intertidal environment, most mangrove plants have evolved effective survival strategies. They have a strong ability to adapt to a hypoxia environment. For example, mangrove plants have developed aerenchyma, expressed as root porosity (POR). The increase of gas space transports oxygen from aboveground parts to roots, constructing an internal way of low resistance for gas exchange between plants and environment (Mano et al., 2006). But significant differences of root porosity have been found in different species and genotypes (Lai et al., 2011). Some oxygen transported to roots can be used towards aerobic metabolism, other oxygen will spread to the rhizosphere. The process of oxygen moved from the aerenchyma to the root is termed as root oxygen loss (ROL) (Colmer, 2003). ROL is an important feature for plants growing in wetland environments and it is one of the adaptive mechanisms which has developed in wetland plants. ROL can affect the uptake of nutrients (Mei et al., 2014), and change heavy metal tolerance in plants (Wang et al., 2011; Wu et al., 2017). ROL varies with the species of wetland plants, probably depending on the

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morphology and physical characteristic of species and rhizosphere oxygen demanded by them (Lemoine et al., 2012; Soana and Bartoli, 2013). Furthermore, ROL is an important physiological factor for iron plaque formation, which is also a reaction of mangrove plants in adapting to the environmental stress (Cheng et al., 2015). Iron plaque can isolate large numbers of heavy metals through adsorption or coprecipitation, so it can interfere with the biological effect of elements and modify the element absorption and transport in plants (Wang et al., 2015).

The response of ROL, root porosity and iron plaque formation of plants in the context of single heavy metals has been reported in various studies (Yang et al., 2014; Wang et al., 2014). However, mangrove plants grow in a fairly complex environment. To date, very little work has investigated the heavy metal tolerance mechanism of mangrove plants under Cd and P interaction. Therefore, our study selected two dominant mangrove species along the China coast (Pioneer specie *Avicennia marina* (Forsk.) Vierh and typical mangrove plant *Kandelia obovata* (S., L.) Yong) as experimental material. The objects of the study were (1) to clarify the effect of P on the Cd distributed in different components of mangrove seedlings under Cd stress; (2) to determine the effect of P on the root oxygen loss, root porosity and iron plaque formation in different mangrove seedlings under Cd stress; (3) to understand the relationship between root oxygen loss, root porosity, iron plaque formation and Cd tolerance. These results will facilitate our understanding of the possible mechanism involved in the adaptability of mangrove seedlings.

2. Materials and methods

2.1. Plant culture and experiment treatments

The mature propagules of two mangrove plants (*A. marina* and *K. obovata*) were obtained from Natural Mangrove Reserve, Fujian, China (23°53′45″ ~ 23°56′00″N,117°24′07″ ~ 117°30′00″E). The propagules were disinfected with 1% $KMnO_4$. Sound propagules were selected to cultivate in pre-washed sea sand. These were irrigated with Hoagland nutrient solution until two pairs of leaves developed. The seedlings of same size were then transferred to 2 L plastic pots containing Hoagland solution. After adapting for two weeks, the mangrove seedlings were exposed to three Cd levels (0, 0.5, 5 mg kg^{-1} $CdCl_2$.2.5 H_2O) and three P levels (0, 30, 90 mg kg^{-1} KH_2PO_4) in nutrient solution culture experiments. The concentrations of Cd and P selected were based on our previous study (Dai et al., 2017). There were three replicates for each treatment. The pH of the solution was adjusted to 6.5 with HCl or NaOH and the solution was renewed every 3 days. The greenhouse condition was 60–80% relative humidity, 25 ± 5 °C temperature and 12 h light/dark at 800–1400 μmol photons m^{-2} s^{-1} (Dai et al., 2017). After one month of treatment, the seedlings were harvested.

2.2. Sample analysis

2.2.1. Determination of the root porosity

The root porosity (POR) was measured according to the pycnometer method (Kludze et al., 1994). About 0.5 g fresh lateral roots were selected and cut into short segments, then dried with bibulous paper. The weight of fresh root was marked as FW. Then the pycnometer was filled with water and weighed marked as TW. After weighing, fresh roots were put into a pycnometer filled with water and weighed again marked as FB. Then roots were taken out of the pycnometer and put in a bottle containing distilled water, vacuumized in a vacuum drier until no air bubbles were seen. After this process, roots were put back to the pycnometer filled with water and weighed marked as FA. The root porosity was calculated according to the different weights of roots before and after the root penetration. The formula was as follows:

$$\text{The root porosity } (\%) = 100 \times [(FA - FB)/(TW + FW - FB)]$$

Where FA = the total weight of pycnometer, water and root after vacuum.
FB = the total weight of pycnometer, water and root before vacuum.
TW = the total weight of pycnometer and water.
FW = the fresh weight of roots.

2.2.2. Measurement of radial oxygen loss (ROL) rate in entire root systems

The rate of ROL of two mangrove seedlings was determined by the Ti^{3+}-citrate oxidation method described by Kludze et al. (1994) with modification. In brief, the roots of seedlings were washed with ultrapure water, then fully immersed in nutrient solution which had already been saturated with N_2. 25 mL configured Ti^{3+}-citrate solution was injected into the nutrient solution with plastic syringe. The solution was immediately covered by a 2 cm thick liquid paraffin layer to prevent the infiltration of O_2 from atmosphere (blank control contained no plants). All operations were carried out in an inert gas box filled with N_2. All the tubes were transferred to the experimental greenhouse. After 6 h reaction, the tubes with or without seedlings were gently shaken, and the absorbance of partly oxidized Ti^{3+}-citrate solution was measured at 527 nm. A standard curve was produced from the relationship between the absorbance and Ti^{3+} concentration. Root dry weight was measured, then rate of ROL was calculated according to the following formula:

$$\text{ROL rate} = c\ (y - z)/G$$

ROL rate = the rate of radial oxygen loss.
c = the initial volume of Ti^{3+}-citrate.
y = concentration of Ti^{3+}-citrate solution in blank control (without plants).
z = concentration of Ti^{3+}-citrate solution after 6 h with plants.
G = the dry weight of plant root.

2.2.3. Analysis of Cd and Fe in iron plaque

The root iron plaque was extracted using the dithionite-citrate-bicarbonate method (also known as DCB) with minor modification (Taylor and Crowder, 1983). The washed roots were put into a triangular flask (about 1 g and cut into 2 cm short segments), and extracted in 20 ml 0.3 mol L^{-1} $Na_3C_6H_5O_7.2H_2O$, 2.5 ml 1.0 mol L^{-1} $NaHCO_3$ with addition of 1.5 g $Na_2S_2O_4$ and shaken for 3 h at room temperature. After shaking, the root segments were washed with deionized water 3 times then the solution was transferred to a 100 ml volumetric flask, diluted with ultrapure water and mixed. A 0.45 μm filter membrane was used to filter. Finally, the concentrations of Fe and Cd in DCB-extracts were detected by inductively coupled plasma mass spectrometry (Agilent 7500cx). After DCB extraction, the roots were dried to a constant weight and the weight was recorded.

Using the following formula to calculate the Fe concentration in iron plaque:

$$\text{The Fe concentration in iron plaque} = 0.1591 * [Fe^{n+}]/\text{root dry weight}$$

Acid digestion was used to detect the Cd content in root, stem and leaf. Blank samples and standard reference material (plant GBW-07603 provided by the National Research Center for Standards, China) were analyzed at the same time. The recovery rate of Cd and Fe were 92–101% and 90–100%, respectively. Finally, the proportion of Cd content in DCB was measured as the following formula:

$$\text{DCB} - \text{Cd}\% = \text{Cd content in DCB/the total Cd content in plant}$$

2.3. Statistical analysis

Data were analyzed by two-way analysis of variance (ANOVA) with a Tukey HSD using JMP 10.0 statistical software (SAS Institute 2012). The relationships between variables were determined via correlation